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ON

BEDSIDE URINE TESTING:

INCLUDING

QUANTITATIVE ALBUMEN AND SUGAR.

BY

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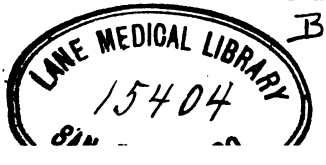

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PREFACE.

TWELVE months have now elapsed since it occurred to me to facilitate urinary examination at the bedside by means of test papers.

Last June I published my notes on the qualitative testing of albumen and sugar by this simple method. Since then the generous encouragement of my professional friends, as well as the growing conviction that the thought I was putting into practice will really contribute—in however small a degree—to the convenience of those whose clinical work lies mainly at the bedside, have stimulated me to push forward that portion of my task left over.

I therefore now bring forward those quantitative methods for the estimation of albumen and sugar provided by the test papers,

which I have found useful. They undoubtedly possess the recommendations of quickness and simplicity.

Quantitative Albumen.—The method proposed for quantitative albumen, I am persuaded, will not merely be found convenient in the daily round, but may supply a want long felt in the consulting room: for the graduated tube with its accompanying standard can define with precision at once the proportions of albumen which can only be gauged by other methods after considerable delay, and even then with less approach to accuracy.

At the present time there is no procedure for the quantitative determination of albumen which can be regarded as at once easy quick, and exact.

Heat and acetic acid, while furnishing the very inaccurate, though generally adopted, method of subsidence, requires time.

Heller's Nitric Acid mode of testing provides merely a general impression of quantity —such as the two deductions of Hoffman and Ultzmann drawn from the density and other physical qualities of the zone of albumen, viz.: "less than half one per cent." "one to two per cent.": and even the plan followed by Dr. W. Roberts, of diluting the urine until *almost* the vanishing point of albumen (·0034 p.c.) with Nitric Acid is reached, though an advance on other procedures, is somewhat tedious from the many consecutive testings required.

Then again, the weighing of the dried albumen after precipitation by heat is not a practical method for medical men; for it consumes too much time, and after all, "the results obtained are only moderately accurate with every care."[1]

The determination by the polariscope is

1. *Urinary and Renal Diseases* by Dr. W. Roberts.

acknowledged, even by its introducer, Becquerel, to be of very limited clinical use—for it cannot be accurately applied to moderately and feebly albuminous urines.

Finally, the volumetric methods (Iodomercuric and Ferrocyanic) have rarely found their way beyond the laboratory, and are by no means so accurate as might be supposed from the nature of a chemical process.

Quantitative Sugar. — A large number of observations have satisfied me that the Indigo-carmine test paper possesses a quantitative power which can be readily and usefully applied in the course of clinical work. So far, I have neither had the time nor the inclination to elaborate a precise analytical method, such as I am persuaded the Carmine test can provide: but I regret this omission the less, because there are already at least three good procedures (Johnson's, Fehling's, and Roberts' differen-

tial density), and the test papers appear to me to afford that practical quantitative information—approximative though it be—which, I think, busy men will be glad to compass without loss of time.

Extension of the Method.—The advantages of this mode of testing are so considerable that one would wish to see them extended beyond the borders indicated by the following pages; and I trust that further enquiry and experiment may permit the application of it to the estimation of other constituents of the urine of clinical interest.

Harrogate,
January, 1884.

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CHAPTER I.

THE OLD AND THE NEW METHODS OF BEDSIDE URINARY TESTING.

1. THE INCONVENIENCES WHICH ARISE FROM CARRYING ABOUT SOLUTIONS OF THE REAGENTS, ESPECIALLY WHEN CAUSTIC OR CORROSIVE.

IT is not necessary to describe the miseries of Nitric acid, and of Fehling's solution: for I have merely to point to dilapidated and spoilt urinary cases, and to the fact that many medical men have been driven by the

objectionable properties of these caustic fluids to give up urine testing entirely during their rounds. Hence, I fear there has often arisen some neglect of an important branch of practical medicine: for it is surely the duty of a medical man to examine the urine of every case—whatever the ailment—and not merely to trust to a suspicion of there being something wrong here and there, and then only to order a sample of the urine to be sent to his house.

But solutions, though physically unobjectionable, are undesirable in the daily round, when compact dry preparations are obtainable, which will remain unimpaired by free exposure, and which can be freely handled, and as harmlessly—to the fingers and the tests—as ordinary writing paper. Fluids must have bottles, which take up room, and the best corking cannot be trusted, especially when the contents are required at every turn in the daily work.

II. Urinary Examination by Test Papers.

About a year ago the idea occurred to me to run the test solutions into chemically inert filtering paper, linen, or other fabric, and after drying, to use the product cut up into test papers.[1] It appeared to me that we

1 Very shortly after the appearance of my first paper on Bedside Urinary Tests (*Lancet*, Jan. 27th, Feb. 3rd, 1883), my friend, Dr. Geo. Harley, informed me, that in 1862, Professor de Luna, of the Central University, Madrid, showed him some urinary test papers. I have since, through Dr. Harley's kindness, examined Professor de Luna's Bolsa Chemica, and the printed instructions contained therein, I found six test papers, viz.: (1) litmus (blue), (2) litmus (red), (3) turmeric, (4) molybdate of ammonia, (5) acetate of lead with nitrate of silver, (6) ozone papers after Schönbein. There were no test papers for albumen and sugar: the detection of which is clinically so important. None of the test papers were of any practical value, so that I think it fair to conclude, that is the reason why what Professor de Luna inaugurated remained sterile. He must, however, be credited with the idea of presenting urinary test-reagents on paper. I need scarcely say that I was totally ignorant of these facts, until my attention was directed to them by Dr. Harley. The concluding

should thus secure the deposition of the reagents in a finely divided and concentrated state; a condition, it was hoped, which would be favourable to such a rapid re-solution of them in the urine as to produce a quick and sensitive action on the constituents sought for. I soon discovered that the pieces of chemically charged paper were, when dropped into the urine, very delicate and cleanly tests; and being in the most portable and compact of all forms for clinical work, and, moreover, affording as good if not even better results than I had previously obtained from the old corrosive

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words of Professor de Luna's memorandum are interesting. "Perhaps further studies, improving this pouch and rendering it more useful in medical practice, will enable us to ascertain with analogous results, sugar, albumen, &c. * * * My intention has been to put into real practice a thought which has occupied my mind for many years. As to the rest time will take upon itself its realization." (Quizà nuevos estudios escaminados á perfectionar está bolsa, haciendola cadu, viz.: más útil en la practica-medica, permitan reconocu con reactivos ánálogos, el azuca, albumina, &c. * * * Yo solo me he propuesto dar forma real á un pensamiento que viene preocupáudome hau algunos anos: lo demás el tempo se encargará de realizarlo).

test solutions, it was not long before I cleared my spoilt urinary case of the latter; and I did so with feelings of satisfaction and comfort.

I should also remark, that the test papers, apart from ministering to the personal convenience of the observer, possess clinical advantages, such as the handy and easy methods which they provide for quantitative estimations at the bedside.

CHAPTER II.

THE QUALITATIVE ESTIMATION OF ALBUMEN.

1. Observations on the working of the old Tests (Nitric Acid and Heat) by the side of the others on the same urines.

No one can form a reliable opinion of the value of any one test compared with that of others, unless he devotes no small amount of pains to a careful observation of the working of the various tests side by side. This rule I have followed since I began this enquiry, and my observations have been very numerous.

In deciding the sensitiveness of the various tests, I preferred to draw conclusions from clinical evidence, rather than from experiments on dilute solutions of ov-albumen or ser-albumen. And, for this purpose, I took a series of urines but faintly impregnated with albumen, presumably derived from the presence of a small quantity of pus, or of blood, or of both, as determined by the microscope. The table of results annotated at the time of every testing is before me. All the urines were acid except one, which was faintly alkaline. The reagents employed were the following:

1. Strong Nitric Acid.

2. Boiling the urine and afterwards adding acetic acid.

3. Saturated solution of Potassium Ferrocyanide, and urine freely acidulated by citric acid, as suggested by Dr. Pavy.[1]

4. Saturated solution of Picric acid, as advised by Dr. George Johnson.

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1 See *The Lancet*, vol. ii, 1882, p. 823.

5. Acidulated Brine, after Dr. Wm. Roberts.[1]

6. Standard solution of Potassio-mercuric Iodide, after Tanret, with, however, this modification — strongly acidifying the urine with citric acid instead of acetic.

The test fluid and the urine were in all the experiments brought into contact, as in Heller's method of using strong Nitric Acid, and the line of juncture was carefully examined for at least five minutes.

Out of the twenty urines Nitric Acid failed to indicate the presence of albumen in sixteen instances, Boiling in fourteen, Acidulated Brine in fourteen, and Potassium Ferrocyanide in twelve; while Picric acid and Potassio-mercuric Iodide gave a distinct and generally a sharply defined ring of precipated albumen in every case.

By the side of these reagents I likewise experimentally tried citric acid dissolved in the picric solution—two drachms to one

1 *The Lancet*, vol. ii., 1882, p. 613.

ounce—and it invariably afforded a more rapidly formed and better defined zone than resulted from Picric acid alone; and, moreover, when the urine and the acidified picric solution were afterwards shaken together, the opacity from precipitated albumen was greater than when the simple picric solution was used. Hence, I conclude that, in detecting small quantities of albumen in urine, the power of Picric acid is quickened and intensified by the presence of citric acid.

The reaction was indicated by varying degrees of rapidity by the different tests; I must name the Potassio-mercuric Iodide, Picric-cum-Citric, and Picric acid as the readiest; and of the three I would, if asked for a preference, decide in favour of the first. I found as a rule Nitric acid, Acidulated Brine, and Potassium Ferrocyanide much slower in bringing to light mere traces of albumen.

The same method of testing (Heller's) was followed throughout these observations for the purpose of securing uniformity in

gauging the results. But I must say with regard to Potassium Ferrocyanide, that I am not quite satisfied as to whether the capacity of this test as an albumen precipitant was in this way fairly put to the trial; for on several occasions I noticed the production of a very slight opacity all through the urine, instead of a well-defined zone. I am, therefore, with this qualification in my mind, inclined to think somewhat better of it than the above recorded number of failures might lead one to suppose.

The outcome of these observations, as well as many more recent ones, suggests to me the grouping of the tests in the following rising order of power to detect small quantities of albumen :—1. Nitric Acid and Boiling. 2. Potassium Ferrocyanide and Acidulated Brine. 3. Picric acid, Sodium Tungstate, Potassio-mercuric Iodide. I have, as a rule, found the members of each group to be nearly equivalent, and confirmatory of each other; and, further, the albumen which Nitric Acid and Boiling discovered was always detected with greater facility by all the other reagents, and those

tests which comprise the third group frequently revealed traces which the others failed to bring to light; Potassium Ferrocyanide and Acidulated Brine certainly took precedence over Nitric Acid and Boiling.

As confirmatory of the foregoing observations, I may mention that I supplied an analytical chemist with some albuminous urine, and he subjected it in the following way to a comparative examination by Nitric Acid and the tests I am introducing in the paper form. After diluting the urine until the albumen was just detectable by the acid, he proceeded to further dilution, when, the reaction failing to appear, the more delicate paper tests still distinctly indicated the presence of the albumen.

The same fact may be illustrated by the following experiment:—ov-albumen is mixed with distilled water, or with urine proved by the test papers to be free from albumen, and is then coagulated out by boiling and acetic acid; after thorough filtration, the clear liquid, though giving no reaction with

strong Nitric Acid, affords a cloud with the test papers.

Undoubtedly the most delicate tests for albumen are the Potassio-mercuric Iodide, Picric Acid, and Sodium Tungstate; and experiment shows that these precipitants are of nearly equal keenness. Then follows in the order of sensitiveness the Ferrocyanic test, which just covers the range of heat and Nitric Acid.

II. The Albumen Test Papers.

Observation having proved the following to be the best and most trustworthy albumen precipitants, viz., Potassio-mercuric Iodide, Sodium Tungstate, Picric acid, and Potassium Ferrocyanide: it was found they were all soluble in water, and could then be evenly distributed through filtering paper, which, when dried, possessed the albumen precipitating power of the reagents unimpaired. The selection of the precipitants was, in the first place, determined by an enquiry which I undertook in order to decide for myself which were the tests of most promise, and the production of them as test papers was quite a secondary matter.

1. *Potassio-mercuric Iodide* was brought forward as an albumen precipitant by M. Chas. Tanret, of Paris.[1] According to my observations it is the most sensitive test known. The precipitate it produces is

1 See *Journal de Connaissances Médicales*, Mai 15, 1872; *also* "*Recherche et dosage de l'albumine dans l'urine*," *Bulletin de Thérapeutique*, 15 août 1877, p. 308.

dense and bulky, and being white, it is moreover much more distinctive than the tawny yellow picric opacity, which resembles the colour of the urine; it likewise coagulates and subsides more readily than the latter. The standard solution, from which the papers are prepared, is the following :—

Grammes.

Mercuric chloride 2·70 } aq destill, 100 c. c.
Potassium iodide 6·64 }

2. *Sodium Tungstate.* According to the Journal of the Chemical Society, for March, 1874, it is stated that this salt had been employed by F. L. Sonnenschein as a sensitive blood test—producing with ammonia a deep green colour, even when the blood was so dilute as not to be recognizable by the spectroscope — and as an albumen precipitant in the presence of acetic, or phosphoric acid. I suppose this important observation has not attracted the notice of clinical observers, for I am not aware of any reference to it in the medical journals in its obvious applications to urinary analysis. On mixing together equal

parts of the saturated solutions of the Tungstate (one in four) and of citric acid (ten in six), and of water. I obtained an albumen precipitant of great delicacy, rapid in operation, and one moreover, so far as I have ascertained, devoid of all objectionable qualities. When merely dropped into the urine, or used after the manner of Heller, it has always quickly revealed the same minimal proportions of albumen as could only be brought to light by the other keenest tests. This combination, when evaporated to dryness on filtering paper, gives results very satisfactory, and the test paper thus produced is, in my opinion, one of the readiest and most sensitive of this series of albumen precipitants.

3. *Potassium Ferrocyanide*, when deposited to saturation on filtering paper, is a very reliable work-a-day test; in my hands it has proved itself almost as sensitive as the other test papers. According to my experiments on peptones, this is the only albumen precipitant of the series which will not throw down peptones as well as albumen. In this respect it may therefore be classed

with Heat and Nitric Acid, which cannot detect peptones. (See p. 51.)

Citric Acid.—All the foregoing reagents are inoperative as albumen precipitants unless the urine is highly acidified; their application should, therefore, be preceded or accompanied by a sufficient charge of acid. For this purpose citric acid is easily made available, when deposited to saturation on filtering paper, and in this form it has afforded me uniformly satisfactory results with all the albumen test papers.

Compound Papers.—Instead of using citric paper separately prior to the reageant paper, it has been combined by a thin layer of rubber with the latter, as a single test paper, in the case of Potassio-mercuric Iodide. The Picric and the Tungstate are likewise presented as compound papers; but in these instances chemical reasons do not necessitate the separation of the citric acid from the reagent: the two are, therefore, united in the same paper.

4. *Picric Acid* can be deposited to saturation on filtering paper, which becomes a

most compact and cleanly vehicle, and which, moreover, quickly delivers its charge to water or urine. Repeated observation has shown me, that when united with citric acid, as in the test papers, Picric acid is divested of most of the objections that have been urged against it. A few drops of albuminous urine instantly turn the bright picric solution, extemporaneously prepared from the test paper, into a muddy one, while the addition of more urine does not dissolve the precipitate with anything like the same readiness as when Picric acid alone is used. Then, again, I have found that Picric acid is apt to fail in albuminous urine when alkaline —a condition which is met by the Picric-cum-Citric test. This test paper is, however, in my opinion, the least satisfactory member of the series.

III. The Mode of Testing.

No urine, while it remains turbid, should be submitted to the tests, or indeed to any test for the detection of albumen: it is best to remove the turbidity by filtration; this simple process, can, of course, be performed anywhere by a piece of blotting paper, or filtering paper carried for the purpose. If the opacity be due to urates it may be removed in a few minutes by a carbonate of soda paper. or at once by warmth, (see p. 41); when phosphatic the turbidity quickly clears up on adding a citric paper.

The nipple pipette (see engraving) is a most useful article at the bedside; it enables the observer to take up a perfectly clean specimen of urine, perhaps otherwise unprocurable, and to examine separately the deposit, or any particular portion of it.

From thirty to fifty minims of the urine are transferred to one of the short test tubes

(see engraving). It is now rendered strongly acid by dropping into it a citric paper, which may be allowed to remain, or may be withdrawn after the interval of a few seconds.[1] It is not now needful to ascertain if the urine be sufficiently acidified—unless it was distinctly ammoniacal, when at least two citric papers should be used—therefore without delay the test paper selected is allowed to fall into it. A simpler plan, and one which answers almost as well, is to drop both the acid and the reagent papers (or a compound paper) into the urine, so that they may fall together to the bottom of the test tube, or the papers may be placed first in the test tube, and then the urine may be run in. Shaking of the tube is not at all necessary or even advisable; it may now be held in a vertical position, or be set aside for a minute. If albumen be present—*e.g.* below a sixth or eighth of a per cent.—a whitish cloud will very quickly gather about the paper, and will collect at

1. Should a precipitate be induced by the acid paper—as will occasionally happen when the urine is highly charged with urates—it will quickly vanish on applying warmth, or on adding hot water.

the bottom of the test tube, or in the lower half of the column of urine: if there be only a trace, the opacity will of course be slight, and will be more readily detected by intercepting the light by the hand, &c., while, in striking contrast, the upper part of the urine will remain clear. If, however, the albumen exists in larger proportions, it will not usually produce a haze, but will coagulate about the paper and will fall from it in clots. The observer will now note the precipitation of it into the lower portion of the urine, where it will gather as a cloud, the density of which will vary according to the albuminous impregnation, while the urine above will retain its transparency. Or, he may now shake the tube, when the urine will become less or more opaque, according to the amount of albumen present. If, on the other hand, the urine preserves its brightness, or if any slight turbidity it possessed prior to the introduction of the test paper is not increased, it may be safely inferred it is free from albumen. The whole proceeding, of course, takes up very much less time than that occupied in reading the

description of it. The reaction is practically instantaneous when the urine has been freely acidified prior to the introduction of the test paper. It is, however, not quite so quickly obtained, though the delay only amounts to a few seconds, when without previous acidification, the compound test papers are used.

Other simple and effective ways of using the papers will suggest themselves to the observer, such as the following:—

(*a*) Those who prefer to develop a zone of precipitation along the plane of contact of a test solution and the urine, can do so by aid of these papers. Two test tubes (see engraving), or a test tube and a wineglass are required. Into one the reagent paper rolled up is placed with about fifteen minims of water, and, without shaking, is set aside, while a similar quantity of urine is put into the other test tube with a citric paper. After withdrawing the latter, the reagent, now in solution, is taken up by the pipette and is allowed to trickle down the side of the tube, in which it will either glide over the urine

or collect below it. After developing the ring, the two fluids may be shaken together, when the albumen will be more largely precipitated as a milky cloud.

(*b*) I am indebted to Dr. S. C. Smith, of Halifax, for the suggestion of a very good and useful method, The papers are bent into a circle, so as to fit within the test tube, and are then pushed some way down: the tube is then filled with the urine. If albumen be present, the whole of the urine below the papers becomes opaque, while that above them remains transparent or unaltered. This is a very pretty experiment, and provides the means of comparing the urine under the influence of the tests with that unaffected by them.

IV. The Keeping Power of the Papers.

None of the papers have been bottled or kept from the air and light during the past year. Still, I cannot discover the least deterioration of their power to precipitate albumen, or any change of colour, or other physical quality. The stability of the papers in the exposure and friction of daily work has now been definitely proved.

V. Fallacies which may be met with.

No one test—as at present known—for the detection of albumen in the urine can, on all occasions be implicitly relied upon: whatever reagent be selected, the results it provides require every now and then to be qualified, or if need be corrected, by other tests or procedures. For instance, Heat alone is clinically valueless: it must be supplemented by an acid, *e.g.*, acetic, otherwise the phosphatic opacity which it frequently produces, cannot be distinguished from the albuminous one; then again, if

the acid or alkaline modification of albumen be present, Heat will fail to bring it to light. Nitric acid is, with the unwary, also apt to mislead, by its liability to precipitate a zone of urates or of urea, hence this test is not always to be trusted unless corroborated by Heat. Picric acid has been shown by Dr. Geo. Johnson to precipitate artificially prepared peptones, and now and then urates also.[1] Potassio-mercuric Iodide and Sodium Tungstate are likewise open to the same fallacies; and though Potassium Ferrocyanide does not throw peptones out of solution, it may, like the other reagents on rare occasions, cause an opacity consisting of amorphous urates.

In selecting an albumen test for ordinary use, the choice, therefore, cannot hinge on the immunity of any particular reagent from fallacies—for there is none that can claim such perfection—but on the comparative absence of them.

On using the albumen test papers, certain precautions have been suggested by ex-

1. See *British Medical Journal*, vol. i. 1883, p. 614 and 859.

experience, which it is advisable to keep in mind, so as to reduce to a minimum the possibility of drawing an erroneous inference from the working of them.

(1) *Highly charged albuminous urines.* When I introduced the test papers, I thought it possible that urines might be met with, so strongly impregnated with albumen, that the instant the paper was dropped into them, a dense film of coagulum might form all over it, lock up the reagent, and thus prevent further precipitation. I have not myself met with an instance of this kind, though the fluid of a hydrocele has provided me with a good example of the fact to which I refer. In this case none of the papers gave a precipitate, but, on withdrawing them, a greasy looking track was left along the inside of the test tube, and they felt soapy; but when the fluid was diluted to about one half, the albumen was thrown down abundantly by all the test papers. On adding Picric acid, either in fine powder or in crystals, to the undiluted fluid, it would not dissolve, but merely caked together, and fell to the bottom, without

precipitating any of the large amount of albumen present; then on diluting the fluid with an equal bulk of water, still, after shaking, the albumen remained in solution: but, on taking a fresh portion of the diluted solution, the Picric powder or crystals threw down the albumen. A similar explanation of the failure of the reaction to that given above applies, therefore, also to this reagent in a solid form—even though finely pulverized—when added to a highly albuminous fluid: namely, the reagent becomes encased in a film of coagulated albumen.[1]

A urine so highly charged with albumen as to cause this behaviour with the tests, is not likely to be met with, without there being present such obvious and unmistakeable evidence of disease as, on the failure of the usual reaction, to cause the observer to dilute the urine, or to prepare a solution of the reagent from the test paper in the second test tube. (See engraving).

(2) *The precipitation of urates* is a comparatively rare event: still it is apt to

1 See the *British Medical Journal*, vol. i. 1883, p. 859.

happen when the urine is concentrated, as in rheumatic and febrile cases, and is then apt to mislead the observer who has not been forwarned. On three occasions I have witnessed the appearance of a voluminous cloud of urates on adding the test papers, and on two of these it followed the use of the citric paper only: in all these instances Nitric Acid produced a dense zone of precipitated urates. I am persuaded it is not safe to rely on the specific differences between the precipitation of albumen and that of urates as they appear to the eye. It is best to resort to the routine employment of warmth (see p. 39) whenever the test papers produce an opacity; then, if wholly due to urates, it will quickly and completely vanish, but any albumen present will remain precipitated.

Experiments and clinical observation have shown me that no other constituent of the urine is likely to be thus precipitated. A strong solution of urea remains transparent after dropping into it the test papers: and an opacity due to phosphates—as when neutral or alkaline urine is boiled—is rapidly

cleared up on adding a citric paper; these salts cannot, therefore, fall out of solution after acidifying the urine.

(3) *Alkaloids* when taken freely by the patient—*e.g.* ten or twelve grains of quinine per diem—may be detected in the urine by the Mecuric and Picric test papers, which then produce an opacity closely resembling the albuminious one. But even in the cold, in the case of the Mercuric test, it can readily be distinguished from the latter by retaining for some time—*e.g.* more than a quarter of an hour—a uniform turbidity, which does not settle; while on the other hand, the opacity induced by albumen breaks up into flocculi much within a minute, and then gradually subsides. Then again, the precipitated alkaloid clears up with heat, or on the addition of alcohol.

Alkaloids in the urine are not thrown out of solution by the Tungstate or the Ferrocyanic test papers.

(4) *Oleo-resins*, *e.g.* Balsam of Copaiba, produce with the citric paper a milkiness, which disappears on boiling, but quickly

returns intensified, even though the urine is still quite warm. Potassio-mercuric Iodide, Potassium Ferrocyanide, and Sodium Tungstate, in the absence of the acid, do not precipitate the oleo-resin, but Picric acid throws it down as a dense opacity, which, like an albuminous one re-dissolves, until an excess of the reagent has been added; it is, however, soluble on boiling, but very quickly reappears.

(5) *Peptones.* (see p. 50)

(6) *Mucus.* The urine, as is well known, not unfrequently contains in solution a good deal of mucus, which, when precipitated, may be readily mistaken for albumen. The presence of this body will, however, in all cases be quickly revealed by the mere use of the citric paper: for, like acetic, citric acid is a sensitive precipitant of mucin;[1] then when a mere trace is met with—a far from uncommon observation — the urine acquires a faint whitish haze, but when it is present in larger quantity, a

1 I first ascertained this fact from observations on pure mucin carefully extracted from ox-bile.

turbidity appears, which has some resemblance to precipitated albumen, for which it cannot, however, be mistaken, for the albumen precipitant paper has not been used. The insolubility of the mucus precipitate by heat distinguishes it from urates. In testing for albumen by the test papers, the presence of mucus is, therefore, revealed at the onset.

(7) I feel some hesitation in mentioning the last precaution I wish to adduce, because it is obviously so insignificant. But I do so, lest there should be such an obtuse observer in the profession, who cannot distinguish between a fine cloud of albumen, and the particles of fluff which may be shaken from the papers; should however, any doubt arise a pocket lens will soon resolve it. When the testing is properly performed—shaking being avoided—there is no fluff to interfere with the detection of the merest trace of albumen.

VI. The Value of Heat in Testing for Albumen.

Inasmuch as the tests introduced as substitutes for Nitric Acid at the beside perform their work satisfactorily without the aid of Heat, the latter is apt to be discarded entirely, as being superfluous. Though observation has shown me, it is but now and then really necessary as a verifier of results, I have nevertheless, found the systematic application of it after the precipitation valuable, and in my opinion it should not then ever be omitted.

(*a*) When small quantities of albumen are detected, it rather intensifies than diminishes the true albuminous haze, though it may not coagulate it:[1] while on the other

1. I have proved by experiment, that when a trace of purified albumen, or a small portion of albuminous urine, is added to normal urine, and precipitated by the test papers, the opacity is slightly intensified by heating, and does not always coagulate, as albumen does when present in larger quantity.

hand, any urates which may have been likewise precipitated will vanish. In working for traces of albumen—such as generally exist in gouty urine—it is easy to prove the superiority of first using the albumen precipitant and then heating, over the comparatively imperfect search effected by boiling only. An albuminous urine is mixed with a non-albuminous one, until boiling fails to precipitate any albumen: to a portion of this mixture the test papers are added, and the haze is warmed, when the opacity —slight though it may be—is obvious when placed by the side of the boiled urine.

(*b*) When albumen is precipitated beyond mere traces, it causes the opacity to collect quickly into coagula, which mass together and float up bodily like clotted cream to the surface—leaving the urine transparent, or only slightly opaque from a trace of albumen that will not coagulate by heat. A better rough notion of quantity can thus be obtained on the spot, than from the density of the precipitate.

(*c*) Heat is likewise a reliable safeguard against the fallacies which may now and

then arise from the precipitation of urates, alkaloids, and peptones.

The Mode of Heating. At the bed-side cleanly heating or boiling is best effected by either of the following ways :—

(*a*) A long wax match, or taper, or candle —the tube being held just above the tip of the flame, when smoking of the glass will be entirely avoided.

(*b*) A small glass-stoppered phial containing spirit: and a cork perforated by a metal tube, which carries the wick—the latter being inserted each time heating is required.[1]

Resumé.—A precipitate induced by any of the test papers is albuminous in at least ninety-nine cases out of the hundred: and is positively so in all cases, when it does not vanish with heat. In practice the only fallacy against which the observer should be always on his guard is the opacity due to urates, which quickly clears up with heat, or is avoided by diluting the urine prior to testing.

1. This convenient arrangement is followed in the urinary cases devised by Mr. Hawksley for carrying the test papers and apparatus.

VII. The Selection of Test Papers.

The first matter to be decided is: should preference be given to the single or the compound papers? The use of the acid and of the reagent apart has undoubted advantages over that of the combination of them in one paper, such as the following:—

(*a*) Any precipitation of urates, of oleo-resins, or of mucus, induced by the acid, can be readily detected, before proceeding to test for albumen by the reagent paper.

Then again, when the urine is turbid from phosphates—not at all an uncommon circumstance—it can be readily clarified by the use of the citric paper.

(*b*) The reaction is quicker.

(*c*) Those who prefer Heat as the reagent, will find the citric paper convenient for the solution of precipitated phosphates. But I think it will be found the best to use the test papers first of all in the search for albumen, because they precipitate all modifications of this body (see p. 49); then the

observer, if he wish to do so, may differentiate serum albumen by Heat, when the solvent power of the acid paper may be called in. Alkaline albumen may likewise be sought for by Heat aided by the previous use of the citric paper; and acid albumen may also be precipitated by it after neutralizing —care being taken not to alkalinize—the acid by means of the carbonate of soda paper. (see p. 79). The separate use of these papers, therefore, enables the observer to utilize Heat, not merely as a detecter of serum albumen, but as a discriminator between the three modifications of albumen.

I therefore prefer to use the reagent paper apart from the acid one.

Then, it may be asked—as I have frequently been—which of the four test papers should be selected as the best? It is very difficult to form an opinion when all do their work with much the same efficiency. In, however, giving my preference, I am but expressing the gist of the opinions of others which have reached me. The Picric paper should be eliminated as the weakest member of the series. The rest are clinically of

nearly equal value, but of the three, I would, upon the whole, decide in favour of the Potassio-mercuric iodide and the Ferrocyanic papers. The latter, by just over-stepping the albumen detecting power of Nitric Acid and Boiling, possesses the great practical advantage in ordinary work of merely compassing one's accustomed area of clinical experience of albuminuria; and the former — though a useful every day test —may prove now and then specially helpful in the study of particular cases, (see p. 45). The reader will also bear in mind that, of all the tests now in use for the detection of albuminuria, the Ferrocyanic is the least liable to fallacies: for, on applying it, one has only to be on one's guard against the precipitation of urates.

VIII. The Clinical aspects of the most sensitive Albumen Precipitants (Mercuric, Picric, and Tungstate).

Though the presence of albumen in the urine is always of pathological significance, the clinical importance of the traces so frequently brought to light by the delicate tests has yet to be determined; probably in the majority of cases it will be found to be small. I am, however, led by clinical observation to regard the most sensitive albumen precipitants as specially fitted to aid enquiry in such directions as the following:—

(1) *Albuminuria of Gout.*—The liability of the gouty to irritation of the urinary apparatus, from the convoluted tubules of the kidneys to the end of the urethra, is well known; and the occasional or intermittent detection of albumen in gouty urine by Heat or Nitric Acid is not an uncommon observation. But the very much more frequent, though perhaps not invariable, presence of a trace of albumen, as shown by these delicate

tests, in such cases, is to me a revelation. It is true it is only now and then proved by the microscope to be of renal origin, and in the majority of cases, it is only due to a small portion of pus, which mingles with the urine as it passes over the irritated urinary mucous membrane. In itself it is, therefore, of but little moment: but though not generally giving rise to symptoms, it points to the advisability of putting a new condition—such as solvent waters—into the existence of the gouty (who should be regarded as a variety of the species *homo*) and then possibly the development of the more important renal disease to which it might lead, may be postponed or prevented. Then again, may not this microscopic form of albuminuria—the tell-tale of gouty blood—be utilized for diagnostic purposes?

(2) *Albuminuria from disturbances of the renal circulation*, as in heart disease, &c. I have sometimes found albumen, not otherwise to be accounted for in such cases, when Heat and Nitric Acid failed to bring it to light.

(3) *The albuminuria of adolescents*, which, according to Heat and Nitric Acid, is so apt to

be intermittent. More watchfulness in the treatment, and more satisfactory results may follow the guidance of the more sensitive tests.

(4) *The complete removal of all traces of albumen from the urine during convalescence from acute renal dropsy*, must be a point of great practical importance, especially with the view of preventing chronic renal disease, which may otherwise supervene years afterwards.

(5) *Every case of Bright's disease does not become established all at once;* it has a beginning in a slight departure from normal non-albuminous urine. Can any one say how often does concretionary irritation of the kidneys, which may induce traces of albumen not recognisable by Nitric Acid or Boiling, merge into chronic renal disease? It is only the systematic examination of the urine in all cases—the apparently healthy as well as the unhealthy—that enables one to pick out here and there the kidneys that are gradually becoming impaired: though there may be no other sign of the fact than the mere trace of albumen brought to light by

these sensitive tests. It is true Nitric Acid or Boiling may, perchance, detect the albumen when it rises in quantity, so as to come within the range of these tests—as it does occasionally; but such an occurrence may not be caught, while the smaller quantities of albumen, which the more delicate reagents can demonstrate, are much more frequently present.

Then again, these tests may be now and then usefully employed in determining the slight and intermittent albuminuria of the early stage of the cirrhotic and amyloid kidney.

NOTE.

THE FORMS OF ALBUMEN AND PEPTONES.

(a) The forms Albumen.

Albumen as ordinarily met with in the urine is serum albumen; and, like that which exists in the blood, it is coagulable by Heat. In some urines, however, it is associated with an alkali, and in others with an acid: then it is no longer thrown down by Heat alone. But these compounds of albumen, as well as serum albumen, are coagulable by Nitric Acid, and by all the test papers; the latter, therefore, cover the same ground as the acid. (See table p. 53).

Then there is Bence Jones' albumen, which, judging from its properties, appears to stand midway between serum-albumen and peptones.

Is there any practical advantage to be derived from discriminating between these modifications of albumen? Inasmuch as the alkaline and acid forms of it are in all probability merely serum albumen accident-

ally associated with an alkali and an acid respectively, is it necessary, clinically, to differentiate between them? Is it not better and safer,[1] to use first of all one of the albumen precipitants that throw down all forms of albumen alike; and then, if it be thought necessary to enquire into the form in which albumen exists in the urine, to appeal to Heat?

(*b*) *Peptones.*

In 1852[2] Mialhe asserted that digested albumen (the peptone of Lehmann) may appear in the urine; and since then the observation has been confirmed by several trustworthy clinical observers. Chemists have distinguished several forms of the soluble and diffusible proteids which pass under the generic term 'peptones': but little is known as to whether one or several varieties of them may be met with in the urine. The

1. I say 'safer' because if heat be chosen as the precipitant of the albumen, acid, or alkaline albumen, even in large quantity, will remain undetected: and to meet this possibility, it will still be necessary to use Nitric Acid, or the other tests.

2. *L'Union Médicale*, 1852.

following general chemical properties isolate them as a family group. (See table p. 53).

(1) Non-precipitation by Heat, Nitric Acid, or by the Ferrocyanic test.[1]

(2) Precipitation by the Picric, Mercuric and Tungstate tests; solubility of the precipitate by heat below the boiling point; and re-appearance of it as a diffused opacity as the solution cools.[2]

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1. The body described by Meissner as A-peptone is "precipitated from its aqueous solutions by concentrated Nitric Acid and also by Potassic Ferrocyanide in the presence of even weak acetic acid." But this, as well as Meissner's B-peptone, are probably not peptones at all. See *A Text-book of Physiology* by M. Foster, M.D., 1883.

2. For 'home' testing the reader will doubtless bear in mind the cupric reactions of peptones in the presence of a strong solution of caustic potash: viz.,

(*a*) A rose or pink tinted (not the mauve coloration from albumen) zone on super-posing a layer of the peptonous urine or Fehling's solution (Dr. Ralfe, *British Medical Journal*, p. 662, vol. i., 1883).

(*b*) To a drachm or two of peptonous urine add a drop of honey or solution of glucose, then a drop or two of solution of copper, and finally Liq. Potassæ: then instead of the blue colour

Since the appearance of the first edition of this publication (June, 1883) I have met met with three cases of peptonuria: two of which were associated with albuminuria.

The table on the opposite page gives the pith of my observations on peptonous urine —derived from digesting albuminous urine —and on the mixture of peptonous with albuminous urine: and on the forms of albumen.

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becoming more intense, it will assume a rich purple tinge, which on boiling will change to a yellow colour without any deposit of the suboxide of copper. (Dr. Dalton first noted this reaction with albuminose. *American Journal of the Medical Sciences*, Oct., 1854; and *A Treatise on Human Physiology*).

THE BEHAVIOUR OF ALBUMEN TESTS ON THE FORMS OF ALBUMEN, AND ON PEPTONES (ARTIFICIALLY PREPARED).

	TESTS.					
	1. Heat.	2. Nitric.	3. Mercuric.	4. Picric.	5. Tungstate.	6. Ferrocyanic
1. SERUM ALBUMEN	+	+	+	+	+	+
2. ACID ALBUMEN	0	+	+	+	+	+
3. ALKALINE ALBUMEN ...	0	+	+	+	+	+
4. PEPTONES (Digested serum albumen).....	0	0	+	+	+	0

Reagent 3, 4, or 5 being used.

The precipitate when heated.

1. Coagulates, and separates from the urine, which remains clear in the cold=ALBUMEN.
2. Clears up entirely, and re-appears in the cold as a diffused opacity, which is soluble by heat, and returns without precipitation in the cold=PEPTONES.
3. Clears with coagulation: coagula settle, and the supernatant liquid becomes turbid as it cools:—the turbidity dissolving with heat and re-appearing in the cold=ALBUMEN with PEPTONES or URATES.

Picric peptonic opaciety soluble in Nitric Acid.....} which do not dissolve
Mercuric „ „ Hydrochloric Acid .....} Urates.

A slight opacity when heated.

1. Soluble, but re-appearing on cooling=PEPTONES or URATES.
2. Persistent—not coagulating or depositing=ALBUMEN.

\+ Indicates a re-action (precipitate.) 0 Indicates no re-action.

CHAPTER III.

QUANTITATIVE ALBUMEN.

I think it will be generally admitted that we are much in need of some ready method by which the quantity of albumen may be accurately determined at the bedside, or in the course of clinical work. The albumen test papers have suggested to me several simple procedures, which promised to supply this desideratum; but I will describe only one of them, because it surpasses the others in the desirable working qualities of simplicity, quickness, and accuracy.

In the first place, it is necessary to select a standard of opacity, which represents a known per cent. of albumen, and then to apply it as the means of determining unknown quantities.

I. The Standard.

When the test papers are dropped into a solution of pure serum albumen of known per cent., and when thereby all the albumen present is precipitated, the density of the opacity produced is seen to vary with the precipitant: being greater, for example, with the Potassio-mercuric iodide than with the Ferrocyanide. This fact is best shown by viewing printed matter through the milky fluid contained in flattened test tubes of uniform thickness (see p. 124): when it will be seen that the different turbidities induce proportionate degrees of blurring of the letters.

The test papers, however, cause an equal amount of opacity when a certain per cent. of albumen is presented to each kind: *e.g.*,

$\frac{1}{10}$ p.c. to the Potassio-mercuric iodide.
$\frac{1}{8}$ p.c. ,, ,, Potassium Ferrocyanide.
$\frac{1}{6}$ p.c. ,, ,, Sodium Tungstate.

It is this uniform opacity — representing these several per cents. of albumen with the

different test papers—which I select as the standard. The starting point of the method is, therefore, derived from known proportions of serum albumen, and not from theoretic data, such as calculations founded on the variously estimated atomic weight of albumen, &c.

The standard opacity has been fixed with some care. Several solutions of different strengths of pure serum albumen were made — the albumen having been dissolved as albuminate of potash. After precipitation of all the albumen by the test papers, the dilutions required by the different tests were made, when all the observations confirmed the uniformity of the opacity thus provided.

II. How is the standard per cent. Opacity made available?

I have found two ways open to this end.

1. In the first instance, I discovered that the perishable albuminous opacity could be exactly imitated by precipitating hy-

drated alumina by means of ammonia from chemically pure alum. This aluminous precipitate closely resembles the albuminous one—for the eye cannot distinguish between the two — and the opacity it furnishes keeps uniformly diffused for at least five or ten minutes. It provides—when properly graduated—a permanent substitute for the albuminous standard; and the two contained in flattened tubes of the same thickness, then produce an equal amount of blurring when placed over printed matter.[1]

2. Lately, however, I have succeeded in dispensing with the permanent standard in the fluid form: and, inasmuch as I obtain results almost as good without it, I am inclined to discard it. A ready means of defining the limit of the selected standard is provided by a piece of opaque glass

1. It is of course important, that the opaque fluid providing the permanent standard should be preserved in a tube having exactly the diameters of the flattened quantitative test tube (see p. 124). Anyone who cares to use it, can procure it, sealed in short flattened tubes of the required thickness, from the vendors of the test papers.

possessing exactly the required degree of opacity. This is fixed over half the printed lines on a card : the other half being left uncovered, so that the observer may view the printing through the flattened tube (see p. 124) containing the albuminous opacity, and compare it with that blurred by the glass.

After the precipitation of $\frac{1}{8}$ or $\frac{1}{9}$ p. c. solution of pure serum albumen with the Potassio-mercuric Iodide paper in the flattened tube, on placing immediately behind it the card on which the red and black lines of various thicknesses are printed (see p. 125), the red lines are more blurred than the black ones, and are scarcely distinguishable from each other ; but on repeating the experiment with $\frac{1}{10}$ p. c. of albumen, they are just discernible : and that is the degree of blurring provided by the opaque glass. Allowing for individual differences of perception, I am satisfied that any observer can in this way quite readily distinguish between the opacities of $\frac{1}{8}$ and $\frac{1}{10}$ p. c. Therefore, the margin of possible error to which this mode of observation is liable, cannot well ex-

ceed the mean between ·12 and ·1; and in determining each per cent. of albumen, this does not allow of a greater variation than ·1·

III. The Principle of the Method.

All the albumen in a measured portion of the urine is precipitated by one of the test papers, and then, when the opacity produced exceeds either the permanent liquid standard or the limit provided by the opaque glass, water is added until the standard per cent. of albumen can be recorded. The data for determining the quantity of albumen in the urine under examination are, (1) the number of times the volume of the urine has been increased by dilution, and (2) the known value of the standard according to the test paper used; and the calculation merely consists in multiplying them together.

IV.

The Selection of the Precipitant.

I am inclined to think the Potassio-mercuric Iodide is the most suitable paper for this quantitative method. It has, besides, the advantage over the others of providing, without calculation, the amount of albumen per cent. in decimal points: for instance, if it be necessary to increase by dilution the volume of the urine five times, the amount of albumen will be ·5 per cent.

V. The Mode of Testing.

The exact dimensions and shape of the test tube are of as much importance in accurately applying this quantitative method, as is the degree of opacity of either the liquid standard, or of the milky glass, which provides the limit to the estimations. The tube to be obtained for this purpose is flattened, possesses a uniform internal thickness of $\frac{3}{8}$in., and is provided with 20 graduations

of 10 minims each.[1] Twenty minims of urine are poured into it; then the test papers are dropped in,[2] and the contents of the tube are shaken, or are made to oscillate up and down the tube while the thumb is held over the mouth for about a minute, when all the albumen will be precipitated. If the opacity completely obscures the printing, dilution may proceed pretty freely, until it is seen that the limit is being approached: then the papers are extracted by the metal clip, and water is added with some care—not more than 10 minims at a time. After each addition the printed card is placed close behind the tube, and, when the lines are obscured to the same degree as by the opaque glass, the proceeding is at an end. I have frequently observed that the last step can be reached by the further dilution of merely 10 minims, which represents only

1. This tube is supplied by the vendors of the test papers. (See p. 124).

2. If the urine is known to be pretty strongly albuminous, it is a good plan to dilute the measured portion of it to twice or three times its bulk, before adding the test papers.

·05 p. c. of albumen; and in case of doubt as to whether the limit has been reached, the addition of this small quantity of water, will, as a rule, resolve it. Just as each observation is being made, the thumb should be placed over the mouth of the tube, and the contents should be gently mixed up without frothing them: the uniformity of the opacity will thus be secured.

If on diluting to 200 minims (the limit of the scale provided by the test tube) the opacity still over-blurrs the printing, 100 minims should be removed, and dilution should proceed either as before (i.e., by adding 20 minims at a time) or by steps of 10 minims. Of course now the latter—not 20 minims as at first—represent the standard value.

If the observer prefer to commence with a larger quantity of urine than 20 minims—*e.g.*, 40—the dilutions which are to represent the per cent. value of the standard should be of course of the same volume (40m).

When the limitation provided by the printed card has been reached, the calculation of the albumen becomes an easy

matter—for the number of times the volume of the urine has been increased by dilution represents so many standard values per cent. of albumen: *e.g.*, on using the Mercuric test paper, if it is found necessary to dilute the 20 minims of urine to 180, the quantity of albumen is ·9 p. c.; 20 minims of urine with the Ferrocyanic test, requiring dilution to 180 minims, contains $1\frac{1}{8}$ p. c.

The printed card carrying the opaque glass, or the alumina standard opacity, is also a useful companion at the bedside when only small quantities of albumen are met with: for by using either, in the ordinary qualitative testing the observer at once can readily and definitely decide the amount; when, for example, the printed lines are too clearly discernible through the opacity, the Mercuric test paper having been used, the proportion of albumen is below $\frac{1}{10}$ p.c.; or when the estimation merely requires the urine to be diluted to twice or three times its bulk, it is $\frac{1}{5}$ or $\frac{3}{10}$ p.c.

VI. The Quantitative Value of each Test Paper.

On submitting 20 minims of urine to examination, the quantitative range of a Ferrocyanic or Tungstate paper is at least 4 per cent., and that of a Potassio-mercuric iodide, now to be supplied, 3 per cent. Inasmuch as these large proportions of albumen are only quite exceptionally met with, one test paper will therefore, cover all the ordinary amounts, and the observer will only rarely require to use a second.

VII. The Quantity of Albumen.

As a rule albuminous urines contain less than 1 p.c.; only now and then the amount rises to 2 p.c.; and it is but a rare observation to find more than from $2\frac{1}{2}$ or 3 to 4 p.c. The proportion in blood-serum is only about 5 p.c.: and, when the urine contains this large amount, boiling completely solidifies it. The general impression as to albumen appearing in the urine in larger quantities than these is groundless.

VIII. The Daily Amount of Albumen Discharged.

As with other quantitative estimations of urinary constituents, so with this, it is the determination of the per cent. in a portion of the urine of the whole day, and the total amount thrown out during twenty-four hours, that is clinically of most importance. When the urine examined is part of the daily yield, and the latter has been measured, it is not difficult to arrive at the total daily loss of albumen in intelligible figures, for it is only necessary to multiply the per cent by 5 to arrive roughly at the number of grains to the fluid ounce; *e.g.*, albumen ·6 p. c., the 24 hours urine 40 oz., (·6 × 5 × 40) = 120 grains daily discharge of albumen.

IX. Estimation of the Albumen by the Round Test Tube.

The smaller of the two round tubes (see p. 127) is available for this quantitative method. The results it provides, though not so precise as those afforded by the flattened tube, are gauged in the same way—each dilution required representing the same standard value.

CHAPTER IV.

THE QUALITATIVE ESTIMATION OF SUGAR.

I. The Detection of Sugar by Means of Test Papers.

All the reagents employed as tests for sugar are either caustic alkalis, or bodies which must be associated with an alkali; but in the latter cases it is by no means necessary to use a caustic alkali. I have, for example, proved by experiment, that carbonate of soda will work quite satisfactorily with Mercuric cyanide, Picric acid, and

Indigo-carmine.[1] I have used all these tests in the form of test paper with good and reliable results: but the Carmine has appeared to me not only the best adapted to this mode of observation, but the least open to fallacies. (See pp. 96—100). Since bringing this test paper forward last May, my experience has corroborated my first impressions of its usefulness and reliability as a test for sugar in the urine, and of its applicability to bedside work: and its capacity to provide without loss of time approximative quantitative information in the course of clinical observation, is a further recommendation.

1. In the case of the Indigo-carmine test carbonate of soda *must* be used instead of a caustic alkali, which discarges the blue colour, even in the absence of a reducing agent, such as glucose. (See p. 100).

II. Indigo as a Test for Glucose.

When casting about for a good and at the same time convenient test for sugar in the urine, I was particularly struck with a fact relating to Indigo; and that was the presence of this intensely blue substance in a colourless state, when associated with glucose or some similar sugar; for instance,

(*a*) When in the sap of living plants (Indigofera and others) it is combined with Indiglucin, ($C_6H_{10}O_6$) which has a chemical formula not far removed from that of Glucose ($C_6H_{12}O_6$).[1]

(*b*) When the dyer mixes Indigo with grape sugar and dilute caustic alkali to produce a colourless solution in which to immerse his fabrics, which acquire a blue colour on exposing them to the air, and

(*c*) When Indigo appears in normal and pathological quantities in the urine, it does

1 Indiglucin in some of its properties resembles glucose; for instance, when heated, it gives off an odour of caramel, it also reduces cuprous oxide from an alkaline cupric solution, and the metals from the salts of silver, gold, &c. It does not, however, ferment with yeast—it only turns acid.

so — according to Schunk — in the form of colourless Indican: a substance which, moreover, as just stated, exists in the woad and other indigo-yielding plants. When this compound is broken up, it is supposed by Schunk, that on the one hand, Indigo-blue (Indigotin), or its isomer, Indigo-red (Indirubin), and on the other, Indiglucin, are set free, according to the formula—

$$\underset{\textit{(Indican)}}{C_{26}H_{31}NO_{27}} \times 2H_2O = \underset{\textit{(Indigo-blue or red)}}{C_8H_5NO} \times \underset{\textit{(Indiglucin)}}{3C_6H_{10}O_6}$$

Almost every clinical observer must have met with ammoniacal urines tinted blue, or violet, or reddish violet; such urines are probably good examples of this reaction; the blue tint arises from free Indigo-blue, and the violet one from a mixture of the red with the blue isomeric forms of Indigo—but the source of this colouring matter must in all cases be referred to the colourless Indican, a normal constituent of the urine, which is apt to be split up into Indigo-blue or red, and Indiglucin. Therefore, it would appear according to Schunk's investigations, that Indigo associated with a sugar, passes from the system in the urine un-

recognised, because it is thus deprived of its colours (blue or red), and it only becomes evident to us when disassociated — the Indican being split up.

It then appeared to me a reasonable question to ask, Can glucose in the urine be made in some way to discharge the deep blueness of Indigo, and thus to tell the tale of its presence? Experiment gave a positive answer: for when Indigo was suspended—it did not dissolve—in a weak solution of soda, or in a stronger one of carbonate of soda, a test solution was obtained, which, when heated with a few drops of diabetic urine, underwent a series of remarkable changes of colour — from blue to green, then to violet, to red, and finally to yellow. I longed to run the liquid containing carbonate of soda and Indigo into filtering paper, and use it as a test paper; because, with carbonate of soda as the alkali, the test papers would have been more durable than with the solution of soda. But unfortunately, after deposition on the paper, the Indigo would not leave it.

III. The Alkaline Indigo-Carmine Test.

My attention was directed to the use of Indigo-carmine as a test for glucose by a paragraph in a work by M. Méhu, which stated that when the Carmine of Indigo is heated with carbonate of soda, and a solution of glucose or saccharine urine, the blue colour is converted gradually into green, then into red, and finally into yellow.[1]

Carmine of Indigo is the sulph-indigotate of sodium—a salt intensely blue and soluble

1. *L'Urine*, par Dr. C. Méhu. Paris, 1880. Since the above was written, Dr. Ralfe has shown me a passage to the same effect, in Neubauer and Vogel p. 73 (Sydenham Society) which I had not previously seen. These authors refer to Mulder, who appears to have been the introducer of this reagent for clinical purposes. I find no reference to Indigo as a test in that invaluable, if not indispensable record of medical progress— Dr. Neale's Digest—or in the works of English authors, within my reach.

(solubility 1 in 120 parts water). Sulphuric acid when heated with Indigo produces the soluble sulph-indigotic acid, which, after combining with a base (such as sodium, calcium, magnesium, &c.), provides us with Indigo as a reagent in a perfectly dissolved state. When carbonate of soda is mixed with a solution of the Carmine the latter is precipitated in a fine state of division; when freshly made and shaken this mixture may pass for a solution much like that of Fehling in colour and general appearance. A perfect solution of a greenish blue tint is, however, obtained on heating the liquid.

IV. The Test Papers Possess Advantages over the Solution of Indigo-Carmine.

The mixture of the Indigo-carmine and carbonate of soda in water, undergoes a gradual change, which renders the test useless; the rich indigo blue slowly gives place to a faded pale green. The test in an aqueous form, is, therefore, not available unless the constituents are kept apart as two solutions. The inconvenience of doing this is obvious, but it is surpassed by that which arises from the necessity to use on every occasion exactly the same porportions of the Carmine and the alkaline carbonate, otherwise—as I have frequently observed—the results of the testings are not comparable. The liquid preparation of the test is valueless as a clinical instrument, and its grave defects must have speedily led to its disuse.

The test papers, however, not only meet these disadvantages, but amplify the powers of the test.

(*a*) Every paper is charged with the same definite quantity of the reagents; it thus provides a uniformity for the qualitative testing, which also, becomes a standard of known value for the quantitative estimation. An additional range of sensitiveness is, moreover, provided by the paper containing a uniform charge of carbonate of soda, when used with the ordinary test papers.

(*b*) The paper furnishes a perfectly transparent[1] alkaline solution of the sulph-indigotate, and all the reagent with which it is charged becomes completely reduced by the sugar, so that in the quantitative estimation the colourlessness of the paper as well as that of the solution will be found to be the guide as to the termination of the completed reaction. When the liquid preparation is boiled it assumes a greenish colour: Méhu,

1 A clear solution cannot be obtained from the papers heated in the London or other hard drinking water. The characteristic re-action of the test is, however, precisely the same.

Neubauer, and Vogel are, therefore, incorrect in giving that hue as the first stage of the reaction of Indigo-carmine with grape sugar. On the other hand, when heat is applied to the test papers in water, a fine true blue solution is obtained, much resembling Fehling; and no amount of boiling will induce a green tint—even though saccharine urine be added. Unlike the solution, the test paper, therefore, provides a clean start for the testing: so that the urine to be examined may, with some saving of time, be added before heat is applied, and the first change of colour, which after ebullition gradually appears, can be safely taken as the earliest step in the reaction.

(*c*) The stability of the test papers is beyond question. The constituents, being dry, remain unchanged; and, when dissolved out of the paper, they furnish a freshly prepared solution at each observation.

V. The Reaction.

The characteristic reaction which indicates the presence of glucose in the urine, arises shortly—*e.g.* within a minute—after the first simmer of the solution prepared from the papers, a drop, or at most two of diabetic urine having been added before the heating. Then a beautiful violet tint suddenly spreads throughout the bright-blue solution; very quickly the violet deepens and passes into purple; this in its turn melts into reddish-purple, which gives place to various tints of red, and these as quickly merge into orange-red and orange, and finally the solution becomes of a straw colour, which remains without further change, though heated ever so long. At this point the paper assumes the same light-yellow colour as the liquid. The complete range of this striking colour reaction embraces all the prismatic colours except green, and the order of the appearance of the successive hues is always the same. The reaction is one of great beauty; for the primary colours are not merely pure

and sharply defined, but all the transitional and intermixed tints pass quickly before the eye in such rich profusion as one rarely sees in nature herself. Now, on shaking the tube the colours return in the inverse order to that in which they appeared. This remarkable thing is not due to cooling, but to admitting the oxygen of the air into the liquid; for the various hues at any stage of the reaction may be caught and retained for days, merely by corking the tubes full of the solution, and the return of the colours, when the test tube is at rest, always appears first at surface, and slowly spreads downwards—so slowly that after putting the solution aside for some hours, at least the lower half will still retain its acquired colour.[1]

1. Inasmuch as the return of the colours is clearly due to oxidation, it will probably be safe to presume that the re-action of glucose on indigo blue is a process of deoxidation. The first stage of the reaction is pretty clearly the conversion of the blue into the red isomeric form of indigo—hence the shades of violet, purple and red; and just that small amount of glucose can be added so as to secure only these steps of the reaction. The second stage is the gradual merging of red into pale yellow—the colour of indigo-white when dissolved in aqueous alkalis.

Experiment has shown that the tint reached in any particular observation depends on the quantity of glucose added to the test liquid—*e.g.*, the reaction may stop at violet, purple, red, &c., and when it thus halts, it can easily be made to proceed to the final stage by adding more of the glucose charged urine—the liquid the while being kept hot. This suggests a principle on which to found a quantitative analysis. The method I employ is a very simple one; it is based on the complete removal of all the colours below the pale yellow: and on the scale of colours when the quantity of sugar is small (*e.g.*, below 5 grains to the ounce).

VI. The Carbonate of Soda Papers.

Test papers charged with a saturated solution of Carbonate of Soda are provided for the following special purposes:

1. *When hard water is used.* I have successfully tested saccharine urine with water highly charged with earthy carbonates, and I do not know that the presence of these salts will ever prevent the carmine reaction; but, merely as a precautionary measure, I suggest the use of the soda paper whenever the water is exceptionally hard.

2. *Excessive acidity of the urine.* The reader will bear in mind that the acids of the urine rob the carmine paper of so much alkali: so that the addition of more than a certain number of drops of urine—varying of course with the degrees of acidity—will at first retard and then prevent the reaction.[1]

1. The reaction of solutions of glucose with the carmine paper alone can be stopped by adding certain quantities of urine of average acidity, *e.g.*, that provided by 40grs. to the oz. by about 40 drops of urine; 20grs. by 20 to 25 drops; 5grs.

Invariably submitting only one drop of saccharine urine to the test paper, and keeping up the heating for not less than two minutes, I have hitherto always witnessed the characteristic display of colours without requiring to use a carbonate of soda paper: so that I am led by observation, to regard the carmine paper as complete in itself for the detection of glycosuria—providing of course the observer uses merely one drop of the urine delivered from the pipette held vertically (see p. 81). But it may be well for the reader to bear in mind that a very exceptionally acid saccharine urine may perchance be met with which may require also the soda paper. I purposely avoided charging the carmine paper with more soda than it now possesses, because experiment showed me it then became too sensitive for a good practical test—one drop of normal urine or of a solution of glucose (gr.$\frac{1}{2}$ to the ounce), for example, then developing a distinct violet in the course of heating for two minutes.

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by 7 drops, &c.: but the reducing power of the glucose is again restored on adding a carbonate of soda paper.

VII. The Mode of Testing.

1. One of the papers should be dropped into the half-inch test tube, and then water should be poured in to the 50m mark; a column of fluid one inch in height and half an inch in diameter will thus be produced, so that the solution of the carmine obtained on boiling will always acquire the same concentration.

2. Heat is now applied (see p. 41), the tube being gently shaken, and boiling kept up for a second or two. The solution will then be quite blue; and, if the water added was soft or distilled, it will be perfectly transparent. Any turbidity observed will arise from the use of hard water: in which case a carbonate of soda paper should be dropped into the solution. The test paper may now be removed, or it may be allowed to remain.

3. Not more than one drop[1] of the sus-

1. The pipette when held vertically will deliver drops of nearly equal size, *i.e.*, about half minim. But, if the drop is allowed to fall from it in a slanting position, it will be larger, and more liable to variation in size.

pected urine is let fall into the tube from the pipette, held in an upright position.

4. The contents of the tube are again freely boiled for a few seconds: then the tube should be raised an inch or two above the flame, and held without shaking, while the solution is kept quite hot, but without ebullition, for exactly one minute by the watch. If glucose be present in abnormal amount, the soft rich blue will be seen first of all to darken into violet: then, according to the quantity of sugar, there will appear in succession, purple, red, reddish yellow, and finally straw-yellow. When the last named colour has been developed, the observer will find the slightest shaking of the tube will cause red streaks to fall from the surface, and to mingle with the pale yellowness of the solution; and further agitation will of course cause the return of purple and violet, and the restoration of the original blue.

The time required for the commencement of the reaction after the boiling of the test liquid, varies in inverse proportion to the amount of glucose present: when the latter is large—*e.g.*, over 20 grains to the ounce—

it will extend only to a few seconds; but when small — *e.g.*, from 2 or 3 grains to the ounce—from thirty to sixty seconds may elapse.

If the urine do not contain more than the normal amount of sugar — *i.e.*, under half a grain to the ounce—the colour of the solution at the end of the heating for one minute, will be unchanged.

Care should be taken during the observation not to shake the tube, or to keep up free ebullition. There is besides another precaution against which the observer, who has no practical experience of this test, should be warned: while keeping the contents of the tube hot, he should not hold the latter up between his eyes and the sky—for then the early colour changes will probably escape observation; but he should keep it below the eye-level, and view its contents by the reflected light of some bright object, such as a sheet of white paper propped up an inch or two beyond the tube, as a background.

The test is as available by artificial as it is by daylight.

VIII. The Results of the Testing by the Side of Fehling's Solution.

In applying the test papers to different urines, I took Fehling's solution as my guide, because it is the best glucose test.

The results of the working of the two side by side were briefly as follows[1]:—

(*a*) On always submitting one drop of urine to the Indigo test, and the presence of sugar being shown, confirmation was invariably provided by Fehling used in the ordinary way.

(*b*) On the other hand, whenever one drop of urine gave no reaction with the test, Fehling's solution did not give the cuprous precipitate.

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1. In all the numerous observations from which these results were drawn, the test paper contained its constituents mixed together, and not kept apart by a layer of rubber (see p. 112); and heat was applied for one minute only. I state these points, because the duplex paper is more sensitive than the other, and the exact time given to the heating is important.

(*c*) On, however, taking more than one drop of urine a different kind of experience was opened up. Then with various urines a violet or purple tint would strike up on the addition of the second, third, or more drops, and Fehling employed in the usual way gave negative results. But I am inclined to think in the cases in which from two to four drops developed the partial reaction, that Fehling, when applied as follows, showed a change which suggested the presence of a very minute quantity of sugar. The urine—either in its normal state or decolourized by animal charcoal — and the solution were mixed in equal proportions, and, it being found the true blue remained intact—not having been turned to green by an excess of the yellow urine—heat was applied, when though no precipitate was visible, the blue quickly turned to a decided olive-green tint, contrasting strongly with the pure blue of the solution held by it in another test tube. In the majority of such instances the transparent green became muddy, either in the act of boiling, or in a few minutes, but, even

then, the clear supernatant liquid, after the subsidence of the precipitate, was of the same green hue, while the others remained free from turbidity. By adding mere traces of glucose, or small quantities of diabetic urine to normal urine, I have repeatedly tried to reproduce these results with Fehling, but, whenever a reaction was induced, by however small a quantity of glucose, I could never secure a permanent transparency of the green colour, for, either while the boiling was proceeding, or during cooling, it invariably became milky. I can, therefore, endorse the statements of Dr. Wm. Roberts respecting the detection of small quantity of glucose in the urine. "The copper solution having been heated to ebullition, and something less than an equal bulk of the suspected urine having been added, the mixture is again raised to the boiling point. * * * If the urine contains less than half a grain per cent. of sugar, the precipitation does not take place immediately, but occurs as the liquid cools, in five, ten, or twenty minutes, and the manner of the change is peculiar. First, the mixture loses

its transparency, and passes from a clear olive-green to a light greenish opacity, looking just as if some drops of milk had fallen into the tube. This green milky appearance is quite characteristic of sugar. By this proceeding one-tenth of a grain per fluid ounce, or less than one-fortieth of a grain per cent., can with certainty be detected."[1] Inasmuch as, according to these observations, glucose only accounts for the green opacity, an explanation of the transparent green is yet to be found.[2] The in-

1. "*A Practical Treatise on Urinary and Renal Diseases, &c.,*" by William Roberts, M.D.

2. I am now inclined to view this obscure matter by the light derived from the following facts. While proceeding to extract from the heart of the ox, kreatin, inosite, and other constituents of muscular tissue which may appear in the urine, I found the clear concentrated solution, after precipitation of the albumen and phosphates, afforded the characteristic play of colours with Indigo-carmine, and a green reaction with Fehling: in the latter case, after separation of a green tinted opacity, the solution remained for some hours olive green and transparent. When the kreatin crystallized out and was re-crystallized, it gave no reaction with either test. The mother liquor, containing the inosite, still reacted as before, and the crystals of inosite dissolved in water, turned undiluted Fehling into a clear olive-green solu-

tensity of the green reaction (whether milky or clear in the cold) was always proportionate to the colour change afforded by the Carmine test papers: and the urines that gave no reaction with the latter below the fourth or fifth drop, either did not disturb the azure blue of Fehling, or merely turned it to a greenish blue shade.

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tion, and reduced Indigo-carmine. Prof. Arthur Gamgee remarks that inosite "does not reduce Fehling's solution, but changes its colour to green." (*A text-book of the Physiological Chemistry of the animal body.* p. 338). Cloetta, many years ago, found that after the subsidence of a green precipitate, the supernatant liquid became blue, and the filtrate was again turned green by heat (Watts' *Dictionary of Chemistry*). This fact I have noted with several urines, which, though remaining transparent for some time after the boiling, let fall a green precipitate over night, and then presented a clear greenish blue appearance: on, however, heating the transparent supernatant liquid, it became again distinctly green, but not quite so much so as after the first boiling with Fehling. Does inosite in small quantity appear in the urine more frequently than is generally supposed?

IX. The behaviour of the Indigo-Carmine, the Cupric (Fehling's) and the Picric Acid Tests when boiled in the presence of various substances.

A test so little known and understood as the Indigo-carmine should be critically examined, before it can be safely admitted as a clinical reagent by the side of Fehling's solution and Picric Acid. In order to obtain a preliminary gauge of its position as a glucose test for urinary work, I therefore boiled each of the following substances —which are either constituents of the urine, or medicines which may appear in that fluid —with it and the other tests.

*The substances marked with an asterisk * reduce the Alkaline Picric Solution.*

I. CONSTITUENTS OF NORMAL URINE.

No reaction with Indigo-carmine or Fehling.

UREA.	HIPPURIC ACID.
*KREATIN.[1]	SULPHATES.
*KREATININ.[1]	LACTATES.
URATES.	OXALATES.
CHLORIDES.[2]	AMMONIA.[2]
PHOSPHATES.	BUTYRIC ACID.
UNOXIDIZED SULPHUR.	

1. Kreatinin strikes in a few seconds a red colour with the cold alkaline picric solution; the reaction soon attains a maximum intensity, though it is quickened and advanced by heat. Normal urines react in the same way with the picric test without heat. A solution of Kreatin or of Glucose produces no reaction in the cold. Thudichum estimates the average daily excretion of Kreatin and Kreatinin (chiefly the latter), as 11·5 grains (*A treatise on the Pathology of the urine*, Lond. 1858, p. 416) or about ½gr. per oz. Neubauer gives the quantity of Kreatinin which passes into the urine in twenty-four hours as from 9 to 20 grains. About ½gr. of Kreatinin in 1 oz. of water gives a reaction with the cold alkaline picric solution very nearly that afforded by the normal urine: but it does not develop so quickly as the latter. Kreatin is not present in normal urine, but Kreatinin is a constant constituent (*A Text Book of Physiology* by M. Foster, M.A., M.D., &c. Lond. 1883). Dr. Ralfe asserts that Kreatinin, when in excess, reduces the cupric salts (*Clinical Chemistry*, 1883, p. 151).

2. Ammonium Chloride (even in very small

Indigo-carmine unchanged, but Fehling reduced.

URIC ACID. OXALIC ACID.

LACTIC ACID.

Indigo-carmine and Fehling reduced.

*UNOXIDIZED PHOSPHORUS.

II. CONSTITUENTS OF ABNORMAL URINES.

No reaction with Indigo-carmine or Fehling.

LEUCINE. TYROSINE.

ALBUMEN: purified Ov-albumen (after Wurtz.)

Purified Ser-albumen.

Albuminous urine free from sugar.

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quantity), ammonium urate or other ammoniacal salts were shown by Dr. Beale to prevent the precipitation of cuprous oxide, when sugar was present in small quantity. These salts do not reduce the Indigo-carmine, and the glucose detecting power of this test is not impaired by the addition of even more than 3 p.c. of ammonium chloride or other salt of ammonia, or of free ammonia to diabetic urine.

After boiling the Indigo-carmine test with albumen, a drop of diabetic urine reacted as freely as in the absence of albumen; and glucose was detected by it in mixtures of any proportions of albuminous and diabetic urines. But when the quantity of glucose was small, while that of albumen was large, Fehling could not discover the former.

PEPTONES.

BILE:[1] non-saccharine bile charged urine and ox-bile added to water in which the test was boiled.

BLOOD,[1] PUS, or MUCUS in non-saccharine urine.

Indigo-carmine and Fehling reduced.

*AMMONIUM SULPHIDE.

Indigo-carmine reduced, and Fehling turned olive green.

*INOSITE.

1. The sugar contained in these fluids may reduce the tests.

III. CARBO-HYDRATES.

No reaction with Indigo-carmine or Fehling.

CANE SUGAR. *GUM (Acacia)
PURE GYCERINE. GLYCYRRHIZIN.
MANNITE. SALICIN.
BOILED STARCH.

Indigo-carmine and Fehling reduced.

*MILK SUGAR. *DEXTRIN.

IV. MEDICINAL AGENTS, &c.

No reaction with either Indigo-carm. or Fehling.

QUININE.[1] BALSAM OF COPAIBA.
MORPHIA.[1] BENZOATE OF LITHIA.
CODEIA.[1] HYPOPHOSPHITES.
ATROPINE.[1] IODIDES.
CAFFEINE.[1] LIQ. PEPTICUS (Benger)
SANTONIN.[1] ETHER.
STRYCHNINE[1] ARBUTIN.[2]

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1. Schwutzenberger asserts correctly (Wurtz's *Dict. de Chimie*) that quinine, cinchonine, and morphia retard the Indigo reaction. But according to my method of testing, any alkaloid added —in quantity never likely to be met with—to saccharine urine, does not interfere with even the quantitative estimation of the sugar.

2. The glucoside of *uva ursi* which passes freely into the urine. (Lewin in *Virchow's Archiv.* vol. 93, June, 1883).

Reaction with Indigo-carmine and Fehling.

*IRON SULPHATE.

*GALLIC and TANNIC ACIDS and infusions, decoctions, &c., containing them.

Indigo-carmine unaffected, while Fehling reduced.

GELSEMINE reacts green with Carmine on admixture, but the colour is not further altered by boiling or shaking (no reduction); whilst it also turns Fehling green, and reduces it on boiling.

CHLOROFORM.
RESIN (B.P.)
JALAPIN.
CARBOLIC ACID.
SALICYLATE OF SODA. *(Green, not reduced).*
[1]CHLORAL. *(Green, not reduced).*

Indigo-carmine merely decoloured or bleached, and Fehling unaffected.

TURPENTINE.

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1. The urine of persons taking chloral-hydrate reduces Fehling's solution, the Bismuth test, and the salts of silver. This reduction is said to be due to uro-chloralic acid. See *L'Urine*, par Le Dr. C. Méhu, Paris, 1880, p. 120.

THE PIGMENTS of urine have not been isolated, and experimented on apart. The following facts, however, disprove of their taking any share in the characteristic reaction of the Indigo-carmine test.

1. The depth of colour of the urines has borne no definite relation to the reaction. In diabetic cases, in which the urine is often very pale, any suspicion of the colouring matter is practically excluded. But in non-diabetic urines—such as those which give an earlier reaction than normal urines and a green colour and milky turbidity with Fehling—it has often happened, that quite pale specimens have afforded a quicker and more developed colour change than the darker ones.

2. Dark urines invariably gave the same reaction after being discolourized by animal charcoal as before.

After perusing this uninteresting record of facts, the reader will be better prepared to estimate the relative position of the Indigo-carmine test by the side of the others.

General results of the testings.

Of the 64 substances experimented with,

Fehling was reduced by			15
Picric acid	„	„	11
Indigo-carmine	„	„	8

The only substances that produced the characteristic play of colours with the Indigo-carmine test papers reacted with both Picric acid and Fehling's solution; they were as follows:

UNOXIDIZED PHOSPHORUS.
AMMONIUM SULPHIDE.

MILK SUGAR.	GALLIC ACID.
DEXTRIN.	TANNIC ACID.
INOSITE.	IRON SULPHATE.

Both the Carmine and the Picric tests were reduced by Inosite: a substance which merely turned Fehling's solution green, but did not provide the yellow or red cuprous oxide.

Of *medicinal agents* likely to find their way into the urine, the only ones which reacted were Iron Sulphate,[1] and Gallic and Tannic acids: which, moreover reduced both Picric acid and Fehling's solution.

1. "Salts of iron, especially, augment very largely the iron of the urine, though the amount passing off in this way is not known." *The Com-*

So far my experiments have demonstrated that the Indigo-carmine test is not reduced by any of the constituents of normal urine, except the sugars—glucose and inosite; or by the one drop of healthy urine prescribed for detecting abnormal proportions of glucose.

It has been suggested to me that *stale urine* — which is the favourite reducing agent of some wool-dyers—may afford the reaction; one of the advantages, however, of this mode of testing is the avoidance of decomposing urines, with which no test for glucose can be trusted. I have, however, observed that the test papers do not produce a reaction with one drop of ammonical urine, or of decomposing albuminous urine: and ammonium sulphide in weak solution (but sufficient to reduce Fehling) used in the same way is equally negative, but, when

position of the Urine, &c., by Ed. A. Parkes, M.D., Lond., 1860, p. 142. I have found the urine of patients taking iron freely is apt to give reactions with the Cupric, Picric, and Carmine tests, suggestive of small quantities of sugar over the normal amount. In this connection the observation of Graevecke, that iron in the urine is always in the ferrous condition, is instructive.—(*Archiv. f. Exp. Pharm., vol. xvii., p.* 466).

more concentrated, it will reduce the Carmine. When ammonia is freely added to diabetic urine, the reaction is not retarded or prevented; and ammonia of itself cannot produce it. But still I would suggest some caution in inferring the presence of sugar in putrifying urine from the reaction with the Carmine test papers.

It is said that *unoxidized or partially oxidized sulphur*, of which about six grains pass into the normal urine in twenty-four hours,[1] will reduce Indigo-blue. I have however, boiled the Carmine test papers with precipitated sulphur, with sulphites, with hypo-sulphites, and with sulpho-carbolates, and the result, after steadily heating for two minutes, has invariably been negative. I very much doubt if, in any case, the small quantity of unoxidized sulphur present in one or even two drops of urine, can react with the test papers: and this supposition will appear all the more probable when one bears in mind that glucose in distilled water in the proportion in which the

1. See *Clinical Chemistry*, by C. H. Ralfe, M.D., Lond. 1883, p. 138.

sugars appear in normal urine—being about four times greater than that of unoxidized sulphur—merely induces the earliest colour change (violet) after heating two drops with the Carmine solution for two minutes.

It has been asserted that all the *carbo-hydrates* when heated with the Indigo-carmine effect the reduction.[1] Guided by observation, I cannot accept this position; for, I find the Carmine solution prepared from the test papers, when boiled and heated with each of the seven carbo-hydrates mentioned on a previous page (see p. 93) remains unchanged. I think it probable, however, that when the sugars of the urine have been worked out and isolated, it will be found, like Picric acid to react with all of them; and it is possible "it may be thus made available for distinguishing between those forms of sugar sometimes present in urine which give no reaction with copper, and which do not readily ferment, and so help to distinguish those cases from true glycosuria.'[2]

It will probably have occurred to the

1. See *Lancet*, vol. i., 1883, p. 877 and 956.
2. *Clinical Chemistry, op. cit.*, p. 155.

reader that the small amount of urine—only one drop—required for the testing by the Carmine paper, must reduce to a minimum the possible intervention of fallacies which may arise from the presence of constituents occurring merely in small quantities: *e.g.*, phosphorus and sulphur (unoxidized or partially oxidized), ammonium sulphide, saccharoid bodies other than glucose.

There is one substance which—though not a constituent of the urine—will discharge the blue colour of the carmine: and that is a caustic alkali—*Liq. Potassæ or Sodæ.* I think it well to mention this, lest the observer using a test tube containing a trace of Fehling's or the Alkaline Picric solution, obtain a reaction with a drop of non-saccharine urine. The caustic alkali converts the blue carmine into a green solution; and on heating all colour gradually vanishes. This reaction is, furthermore, unlike the characteristic one afforded by glucose, in that any remaining colour after heating slowly fades away on shaking the contents of the tube, and when the solution has become quite colourless no amount of agitation will restore the colours.

X. The Clinical Advantages of the Indigo-carmine Test Papers.

The high position of Fehling's solution as a test for the glucoses in urine cannot be questioned; but for two disadvantages which belong to it—the liability to change on exposure to light and air, and the caustic properties which condemn it for bedside or out of door work—no one would desire a substitute. It has undoubtedly yet much sound work to do, and I have no wish to suggest the dismissal of it from our service. Still, like every other urinary test, it is not equally good all round. Where it is weak and apt to fail, the Indigo-carmine test, as here presented, appears to me to supply useful supplemental aid—apart from other clinical advantages.

1. Sugar in small quantity along with much albumen, may be overlooked by Fehling. As a rule, the search for sugar by this test in albuminous, bloody, or purulent urine, should be preceded by precipitation of the albumen and filtration. These procedures

are, however, unnecessary when the Indigo-carmine test papers are used; for it has been proved by repeated observation, that they will detect sugar—in any proportion, and as readily as in ordinary diabetic urine —in the presence of albumen, peptones, blood, pus, &c.

2. It is well known that uric acid will reduce Fehling's solution: but it has no reaction with the Carmine test. The latter is unaffected by urates: the former is, however, apt to give with them a cuprous precipitate, which may easily lead to a false inference. On this point Prof. Cameron, of Dublin, gives the following very useful caution in applying the Cupric test. "I occasionally find urine with a very high specific gravity, and with a, so to speak, diabetic appearance, to be quite free from sugar. On several occasions, in specimens of urine believed to contain sugar, I could not detect a trace of that substance. A few months ago I examined the urine of a man who had been treated for diabetes. The urine had a specific gravity of 1035, and, on being boiled wirh Fehling's solution,

it gave a copious precipitate of Cuprous oxide. There was something in the appearance of the precipitate, and in the slow way in which it made its appearance, that led me to suspect that it was not produced by sugar. This proved to be the case, for on treating the urine with yeast no carbonic acid (save a mere trace) was evolved. The presence of large quantites of urates in urine causes a brown precipitate with Fehling's solution. The urates, even when abundant, do not always separate as the characteristic 'brick-dust.' I have found very large quantities of urates of ammonium in urine which remained clear on standing, but which gave a brown precipitate on being boiled with Fehling's solution." *(Dublin Journal of Medical Sciences*, April, 1883*)*. In such cases the clinical value of an appeal to the Carmine test must be apparent: for it will in a ready and simple way obviate the fallacy which makes the cupric test untrustworthy.

3. Dr. Ralfe has suggested (see p. 99) that it may prove a valuable supplement to the other tests for sugar, by virtue of its power to detect the saccharoid bodies which

may appear in the urine that do not react with copper, or that do not readily ferment. The reader will, however, bear in mind that hitherto observation has shown that such bodies—*e.g.*, Dextrin, Lactose, Inosite, —but rarely appear in the urine, and that when the reaction is obtained with the Carmine the presence of glucose may be pretty safely inferred.

4. The capacity of the Carmine test papers to afford information as to the approximate quantity of sugar in the course of the search for this body (see Chapter v.) is a valuable working property; for it enables the practitioner at once to form an estimate of the cases of glycosuria examined for the first time, and of the effects of treatment—so far as these can be gauged by the amount of sugar excreted.

5. The stability of the handy and cleanly Carmine test is an unquestionable gain.

CHAPTER V.

QUANTITATIVE SUGAR.

I. The Indigo-Carmine Test Paper Provides Quantitative Information.

A most desirable property of a qualitative test at the bedside is the power to furnish a good notion of the coarser variations of quantity; for this knowledge obtained on the spot must often be of greater utility than even the discovery of finer gradations by methods of precision, which are not available beyond the consulting room, and necessarily require time for their application. It is this practical quality which emphasizes the adaptability of the Carmine test paper to bedside work. But inasmuch as almost every saccharine urine provides pretty uniformly the

complete reaction — the final pale yellow being nearly always reached—the observer will scarcely be prepared to regard the test papers in a quantitative light.

A little observation will, however, soon show that this aspect of them is not only capable of verification, but can be readily applied in practice.

When the attention has been directed to the degree and the rapidity of the reaction with different saccharine urines, great variations are to be detected. In some, for instance, it begins immediately after the drop of urine has fallen into the hot solution, and is perhaps completed in half a minute; while in others the commencement of it is delayed for twenty or thirty seconds, and pale yellow is not reached until even two minutes have elapsed; and in still others at the end of the prescribed time for heating—120 seconds—the colour developed is perhaps only red, purple, or violet.

The cause of all this variability is found to be the different proportions of glucose present. This fact has been proved not only

by quantitative estimations by Fehling's solution, but by dissolving glucose[1] in normal urine, and in distilled water, and submitting —as with saccharine urine—one drop of each solution to the test. The reaction—both in the degree it attained in the specified two minutes, and the rapidity of it—was always proportionate to the amount of glucose. For instance, less than five grains to the ounce would not develop the final colour — pale yellow — at all within two minutes, while 10 grains did so within one minute, and 35 or more grains within thirty seconds.

Glucose always afforded the same reaction, whether dissolved in urine or in distilled water. I therefore conclude that none of the non-saccharine constituents of the urine are causes of variation. Excessive acidity of the urine may, however, in some cases diminish the reaction; but, inasmuch as from ten to twelve drops of normally acid urine are required to appreciably retard the reac-

1. What is generally sold as 'pure' glucose contains, according to my observations, from 25 to 50 per cent. of impurity.

tion of a solution of glucose (20 grains to the ounce) on the Carmine paper, it is pretty clear that the acidity of a urine must rise considerably over the average before it can become a source of disturbance. But, inasmuch as saccharine urines are highly acid, and more particularly so after the lapse of a few hours, it will be as well to eliminate this possible cause of variation of the reaction, by adding on all occasions a carbonate of soda paper to a definite portion of the urine to be examined.[1] (See p. 111.)

On diluting saccharine urines further evidence of the quantitative power of the test papers is apparent, the reaction being delayed or rendered incomplete—as the case may be —in proportion as water is added to the urine. (See p. 117).

1. There is sufficient soda in each Carmine paper to neutralize at least eighty minims of urine of average acidity.

II. The Test Paper.

The ordinary Indigo-carmine paper is available for the quantitative determinations. It furnishes a good general notion of all the proportions of sugar to be met with; but on testing with the urine undiluted it is more particularly applicable to the estimation of the smaller quantities. It is, however, equally useful in the determination of the larger amounts when the urine is diluted.

I have been in the habit of using a test paper four times the strength of the ordinary one for the discrimination of the larger proportions of sugar in undiluted urine; but I have decided not to bring it forward, lest by doing so I should be complicating the simple method of testing as it now stands, without securing at the same time any advantage over diluting the urine, which is equivalent to providing the quantitative power of the strong paper.

The test papers hitherto made have not been prepared with a view to their quantitative application, and have not been cut so even in size as is necessary for this purpose; but I am assured that henceforth special care will be taken to secure the required uniformity. Observation has shown me that slight variations in this respect are of no practical importance; and that the quantitative results are determined chiefly by altering the proportions between the carmine and the alkali, rather than by adding more and more of the materials in the same quantities.

A large number of observations have shown me, that it is better to keep the constituents apart by a layer of rubber. This arrangement increases the sensitiveness of the test paper, and improves its quantitative capacity. The data which follow have been provided by the 'duplex' paper. When this test paper is heated in water, the rubber separates itself from the two papers, and rolls up to the surface out of the way, and the constituents of the test pass completely into solution.

III. The Mode of Testing.

The steps previously described (see p. 76) are to be followed, but with more care in certain particulars.

1. The water to be used should be distilled or soft.

2. The quantity of water to be added should not exceed the 50$\mathfrak{m}$ mark on the half-inch test tube. A wider tube than this should not be used.

3. Twenty minims of the urine to be examined are shaken with a carbonate of soda paper in the larger test tube, which is then set aside.

4. The observer should select, if possible, daylight, and he should place some light coloured object close behind the tube, so that he may view the colour changes distinctly by a bright reflected light. The disappearance of red is, however, perhaps most easily detected by holding the tube against the sky.

5. Before the testing is begun the observer lays before him his watch—a centre seconds is by far the best for the purpose. Time is to be accurately estimated by the seconds hand.

6. Immediately after the paper—which is allowed to remain—has been boiled, when the carmine has well passed into solution, and when the liquid is quite hot, one drop—not more—of the urine from the larger test tube is delivered from the pipette held vertically,[1] and the exact time by the seconds hand of the watch is noted.

7. The solution is then well boiled up for about 10 seconds, and the tube is raised a few inches above the flame, and is very steadily held in that position; but on the slightest ebullition occurring, it is raised still higher. The prevention of simmering during the course of the heating is a most important

1. I am aware that a drop is a somewhat variable quantity: it is, however, I think preferable—in handiness and practicability—to the minim, the accurate measurement of which requires well trained and reliable eyes and fingers, unfortunately not possessed by all; and the variability of the drop, when discharged from the pipette

precaution towards obtaining reliable comparative results. The slightest shaking of the tube—especially towards the end of the reaction—should be avoided.

8. If the complete reduction, indicated by pale yellow, is not effected within two minutes, the heating is kept up for the whole of this period.

The carbonate of soda paper should on no account be used along with the Carmine test paper.

From the time of dropping in the urine the observer should specially note the colour of the solution at the close of

(*a*) Thirty seconds.

(*b*) One minute.

(*c*) Two minutes. (See diagram p. 116).

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held in a vertical position, is not so great as to disturb the conclusions of the text. Those who prefer a more precise method will find it convenient to take 10m of the urine, and make up with water to the 100m mark on the larger test tube, add a carbonate of soda paper, and use 5m at each testing.

IV. Quantitative Data.

The quantitative information to be derived from the test paper is obtained from submitting to it the urine, (1) undiluted, and (2) definitely diluted.

1. The Urine undiluted.

The data provided fall into two sections: according as the reaction is complete or incomplete at the expiration of the period prescribed for heating—*two minutes.*

(a) The reaction is incomplete.

When the final colour change—pale yellow—is not developed, there are less than 5 grains of sugar to the ounce, or under 1 per cent.

Any vestige of red tinging the yellow can be distinctly seen, when the tube is held *without any shaking* about an inch before a piece of white paper: on, however, placing it immediately against the latter, a

trace of red may still be detected — to remove this requires not less than 10 grains to the ounce.

When sugar is present in smaller quantity than 5 grains to the ounce, the colour of the solution at the end of the heating for two minutes represents definite quantities.

Colour.		*Grains to the ounce.*
Violet[1]	=	about 1.
Purple[1]	=	„ 2.
Red	=	„ 3.
Reddish Yellow	=	„ 4

If violet does not appear within half-a-minute, there are less than 2 grains of glucose to the ounce.

If the solution, however, retains its blueness for twenty or thirty seconds, and then during the course of the first minute becomes violet and purple, the quantity of sugar is about 2 grains to the ounce: but if the violet appears at the close of the

1. The casual observer is apt to confound violet with purple: but the colours are quite distinct—the former having blue, and the latter red, as the predominating hue.

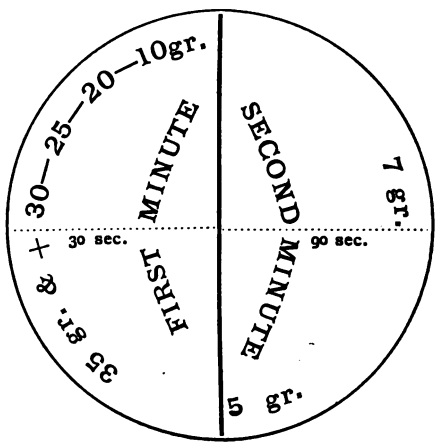
boiling up for ten seconds, there are *at least* from 4 to 6 grains to the ounce.

(*b*) *The reaction is complete.*

The time required for the full development of all the colours is determined by the amount of sugar.

When straw yellow is reached in

	Grains to the ounce.
½ a minute there are about	35 grains or more.
1 „ „ „ „	10 „
2 minutes „ „ „	5 „



The observer should carefully note the rapidity of the reaction in the course of the first minute. If at the close of the first half of it the solution is reddish, the quantity will be less than 35 grains to the ounce: but if it is pale yellow, the amount will be larger than this. If at the termination of the minute a red tinge is still apparent, the proportion will be under 10 grains to the ounce, or below 2 per cent.

2. The urine definitely diluted.

When the reaction with the undiluted urine is completed within one minute only a general conception of the quantity of sugar can be provided by the test paper. But the information thus obtained is a useful preliminary to the acquirement of a more definite idea of the amount which can be attained by the further testing of the urine methodically diluted.

The principle of the proceedure is to take a measured portion of the saccharine urine (20m) and to dilute by the same volume (20m) of water at a time, until at last it is found that at the conclusion of the heat-

ing for a definite period, (either one or two minutes) the colour developed is no longer pale yellow, but has a distinctly red tinge; the approximate amount of sugar is then arrived at by multiplying the number of times the volume of the urine was increased on the previous dilution, either by 10 or by 5 grains to the ounce, according as the time selected for heating extended to one or two minutes. In this way, when urines are found to contain more than these amounts of sugar they are uniformly reduced to them; and the dilution is continued until it is clear the limit has been overstepped —a reddish hue remaining at the close of the procedure.

The selection of one or of two minutes for the heating, determines the gradations —in the former case 10 grains to the ounce, or 2 p.c.; in the latter 5 grains, or 1 p.c.—according to which the amount of sugar is to be estimated.

(a) The solution to be heated for one minute.

On returning the urine contained in the pipette to the large test tube into which

20m were delivered (see p. 111), the observer adds the same measure of water. If, however, on testing the undiluted urine he found the yellow to appear at the end of half a minute, he may at once dilute the urine to three times its volume (second dilution), otherwise he will only add 20m of water, and re-test. If, at the termination of the minute the yellow still appears, the contents of the pipettte are returned to the test tube holding the diluted urine, and a further addition of 20m of water is made. The testing is repeated; but, when it is evident the complete reduction of the blue carmine is not accomplished at the expiration of one minute, the procedure is at an end.

On putting this method into practice under the guidance of Fehling's quantitative determination, I find that when the testing by the Carmine paper affords a reddish yellow tint the value of the last dilution should be counted as five grains; which amount should be added to the 10 grain values of the previous dilutions: but when the final trial provides a more decided red colour the calculation should only include the previous

dilutions. For example: a urine containing according to Fehling 7 p. c. of sugar, or about 36 grains to the ounce, on the second dilution, gave at the expiration of the minute a reddish yellow reaction; therefore the amount was $3 \times 10 + 5 = 35$ grains to the ounce. Another urine, shown by Fehling to contain 6 p. c. of sugar, or about 31 grains to the ounce, just reduced the Carmine on the second dilution, but on the third it failed in doing so, and the solution, on being heated for the minute, merely acquired a redness that could not be mistaken for yellow; the calculation was, therefore, $3 \times 10 = 30$ grains to the ounce.

The determination of sugar by this shorter method does not usually require more than three test papers in all (often two will suffice), and the expenditure of more time than five minutes: and, moreover, the quantitative information it provides is, as a rule, sufficient for most clinical requirements.

(*b*) *The solution to be heated for two minutes.*

The observer, guided by the results of the preliminary testing of the undiluted

urine, and keeping in mind the smaller scale (5 grains to the ounce) which the heating for two minutes requires, at once dilutes the urine more freely than when he selects the larger gradations: if the reaction was completed in half-a-minute he adds five volumes (100m) of water to the one (20m) of urine: or if only in the course of the second half of the minute, he dilutes the urine with two volumes (40m) of water.

The testing proceeds as before: only the dilution may advance by two volumes of water at a time, if the complete reduction continues to take place before the first minute expires; and thus the observer will economize his time as well as the test papers.

This method has afforded me very satisfactory results, and it has always appeared to me, that the reading of the red reaction, which indicated the over-stepping of the limit (namely 5 grains to the ounce) provided by dilution, was somewhat more definite than when the wider scale was followed.

Conclusions.

1. The Indigo-carmine test paper enables the clinical observer to discriminate between the glucose charges of different saccharine urines in the course of the mere qualitative testing.

2. It provides definite, though only approximative, quantitative data; sufficient, however, for most of the clinical requirements of the practitioner.

3. The method proposed for its quantitative use exacts but a small expenditure of time—only a few minutes—and merely that careful attention to a few essential details, and the ordinary skill in observing and manipulating which it is rightly presumed that most medical men possess: and, moreover, it requires for its execution but the simplest apparatus, and consequently can be applied efficiently away from the consulting room.

CHAPTER VI.

Apparatus required for the Determination of Albumen, Sugar, and Specific Gravity.

The adaptability of the method of urinary examination by means of test papers to clinical work outside the consulting room, is not merely indicated by the portability of the test papers themselves, but is further shown by the simplicity and compactness of the apparatus required for their qualitative and quantitative application.

I. Qualitative Albumen and Sugar.

The observer only needs—

1. *A short half-inch test tube.* (Fig. 1).

2. *A nipple pipette.* (Fig. 3). In testing for albumen this useful little instrument may enable one to avoid sediment; and in the search for sugar it provides — when held upright—a uniform drop of very nearly half

a minim;[1] it is also a measure of the 20m dilutions required for quantitative albumen and sugar. When not in use, the tube having been pushed up within the rubber nipple, can be conveniently packed along with the metal clip within the test tube. (Fig. 5).

3. A metal clip used as a tube-holder during the boiling and heating required by the testing for sugar, and for drawing out the test papers, &c.

II. Quantitative Albumen

1. Test tubes. The observer may select either the flattened[2] or the small round tube, (Fig. 1). The shortness of the latter only allows the urine (20m) to be diluted 6 times: therefore, when there is more than ·6 p.c. albumen, either the urine should be diluted with an equal bulk of water in the larger tube (the data obtained being doubled) or

1. The pipette, when held in a vertical position, and charged with 20m of water, should deliver from 36 to 38 drops.

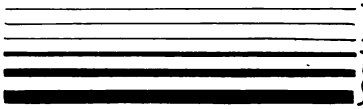
2. I prefer the flattened tube for home observation; for upon the whole, its results are more definite and accurate than those afforded by the round tube.

the scale (2 p.c.) provided by the thick lines (see below) should be selected.

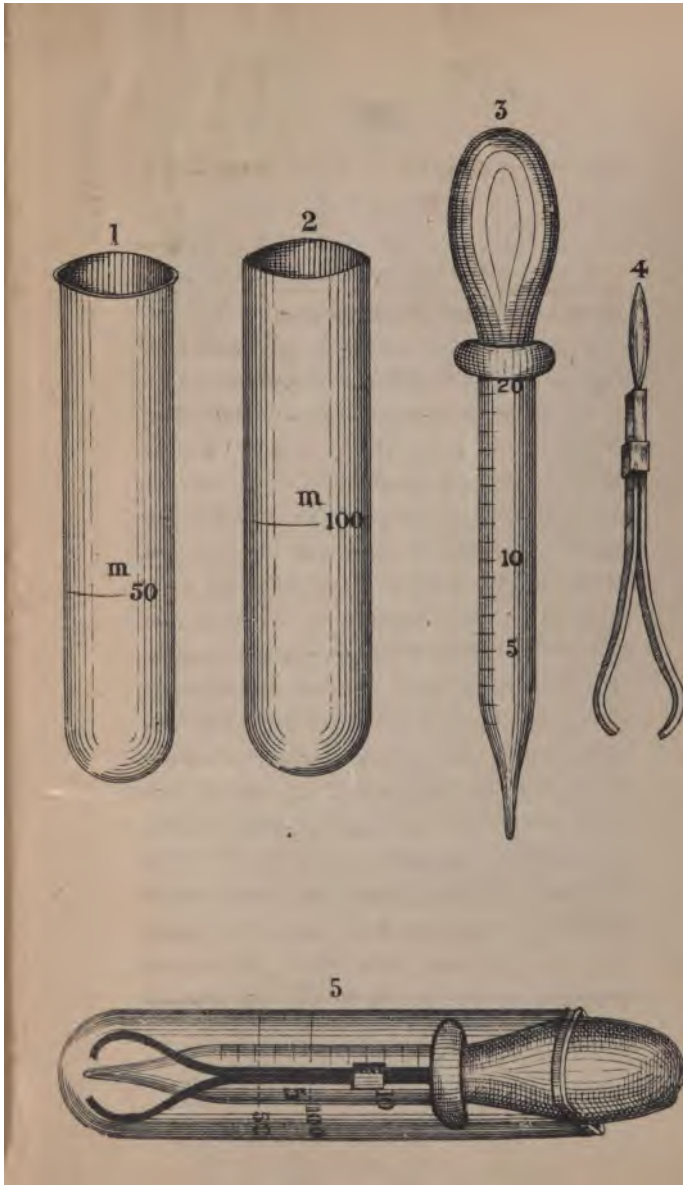
2. The standard opacity is provided in a permanent form by: (*a*) *alumina* precipitated in an oval tube; and (*b*) *opaque glass* fixed on one half of a card on which lines are printed. Observation has distinctly shown that lines are better definers of the limit when dilution should cease than other forms of printing. I have selected two kinds. (1) *Fine lines*, which, on using the 'duplex' Mercuric-iodide,[1] do not come into view until the opacity has been so reduced as to represent $\frac{1}{10}$ p.c., and are then obscured as shown by the permanent standards; but become too distinct on diluting to $\frac{1}{11}$ p.c. (2) *Thick lines*, which are just discernible when the opacity is equivalent to ·2 p.c., and provide a good general effect—being distinctly over-blurred by opacities greater than $\frac{1}{10}$ p.c., and insufficiently obscured by those of smaller amounts; they will be specially useful when the eyesight is not particularly acute. I have lately proved

1. This is the best test paper for quantitative albumen.

that this arrangement is better than that of the red lines mentioned on p. 58.



It will be found, however, that either of the permanent standards is useful merely as a temporary guide to the eye: for, after a little practice the observer will learn to use the printed lines correctly without it. I am satisfied that, with $\frac{1}{9}$ p.c. opacity, the fine lines cannot be detected through the centre of the oval or round tube by keen sight aided by a bright light, such as that of the sun, or of a lamp, but are readily observed in ordinary light by anyone when the opacity is reduced to $\frac{1}{10}$ p.c. The observer may, therefore, come to rely on merely discovering the limit of visibility of the lines viewed through the opacity with a good light—either transmitted or reflected. The lines are printed on a card, partly covered by the opaque glass; and on paper—for those who can dispense with either permanent standard, or who prefer to use the alumina one.


1
m
50
2
m
100
3
20
10
5
4
5

III. Specific Gravity.

A good many observations on the working of the small urinometer provided for bedside use have caused me to discard this fragile and inaccurate instrument. The errors to which it is liable—often inducing variations of more than 5 degrees — are mainly caused by the different quantities of urine put into the trial glass, and by the temperature of the urine. It is only in name and appearance an instrument of precision. In its stead I greatly prefer a selection of six urinometer beads (1010, 15, 20, 30, 40, 50), each distinctly marked in black letters, and small enough to work satisfactorily in the large round test tube (Fig. 2) as a trial glass.[1] The information they provide is reliable and decisive, and is sufficiently ample for all practical purposes. Besides, they are handy and portable, and are not at all liable to fracture.

1. These beads were made for me by Mr. Hawksley, 357, Oxford Street, London, W.